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Studies on spin labeled ribonucleic acids encapsulated by viral proteins

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ABSTRACT

Spin labeled poly rA (sl-poly rA) was encapsulated by the coat proteins of two plant viruses having different morphologies: TMV, a rigid rod and CCMV, an icosahedral sphere. Electron microscopy showed that the resultant particles were morphologically similar to the parent virus from which the coat protein was obtained. Encapsulation produced progressive immobilization of the spin label. The motion of the spin label attached to TMV-sl-poly rA appears anisotropic with a correlation time about the long axis of approximately 5×10^{-6} sec. Exogenous nuclease had no effect on the epr spectrum of this nucleo-protein complex. The epr spectrum of CCMV-sl-poly rA was isotropic with a correlation time less than 5×10^{-7}. CCMV-sl-poly rA was partially degraded by T_2 ribonuclease. Theoretical calculations of correlation times for the motion of the nucleo-protein particles were similar to the experimentally derived values suggesting that the nucleo-protein particles are tightly packed with little potential for internal motion.

INTRODUCTION

While spectroscopic studies on the nucleic acid conformation in simple systems have been widely exploited, such studies in complex systems are hampered by interfering signals arising from the environment. In certain cases this problem can be circumvented by attaching a molecular reporter group, such as a nitroxide spin label, to the nucleic acid. The resultant electron paramagnetic resonance (epr) signal is observed readily since most biochemical or biological systems possess few free radicals. Previous studies on the properties of spin-labeled nucleic acids in solutions from our laboratory (24) and from others (1a-1g) have indicated that nitroxide spin labels accurately monitor localized conformational events. We have now extended these basic studies to a more complicated system, the encapsulation of spin labeled poly rA by the coat proteins of two plant viruses differing greatly in their morphologies.

The viruses, tobacco mosaic virus (TMV) and cowpea chlorotic mottle mosaic virus (CCMV), have simple compositions and regular, repetitive substructural groups. The viruses can be disassembled into capsid protein and RNA, and reassembled *in vitro* using the capsid protein and the original RNA, RNA from

different viruses or synthetic polynucleotides. These two viruses also differ in overall shape suggesting that possible differences in their respective nucleic acid environments might be monitored by epr.

Considerable information is available concerning the conformation of the RNA inside the rigid rod-shaped tobacco mosaic virus, TMV (2,3,4,5). The single-stranded RNA is arranged as a helix with the same pitch as the helix formed by the coat protein monomers (6a). The RNA phosphate backbone is 40 Å from the center of the helix and the RNA helix appears to be puckered(6b). The planes of the RNA bases apparently are parallel to the long axis of the particle (7,8) and three RNA bases are in contact with each protein monomer (9). The RNA inside the virus particle is hyperchromic compared to its form in 0.1 M salt solution (10,11).

The arrangement of the RNA inside the spherical virus (12,13), cowpea chlorotic mottle virus, CCMV, has not been defined. The genomic RNA is single-stranded and is present as four species (14). Particles of CCMV contain one or two pieces of RNA with a net molecular weight of approximately one million daltons (15). The virus exists in its native icasohedral form at pH 5 while at neutral pH in the absence of magnesium, the virus exists in a swollen and probably spherical form.

In this paper, we show that when added to the viral proteins under assembly conditions, spin labeled poly rA (sl-poly rA) was encapsulated into pseudo-virions. The dynamics of encapsulation could be followed by epr. The accessibility of the encapsulated sl-poly rA to the external environment was infered from its susceptibility to exogenous ribonuclease. Epr analysis of the pseudo-virions together with the ribonuclease susceptibility studies have allowed for the *in vitro* observation of the physical arrangement and environment of the nucleic acid bases in viral particles.

METHODS

Preparation of Viruses

TMV, common strain U1, propagated in Nicotiana tobacum Maryland (var. Turk) in a controlled environment plant room was isolated by repetitive differential centrifugation. Millipore filtration provided additional purification (17).

CCMV, originally obtained from J. B. Bancroft, University of Western Ontario, and propagated continuously in Vignal unquiculata (L.) Walp (var. Blackeye) in a controlled environment plant room, was isolated by polyethylene glycol precipitation (17) and differential centrifugation (Bancroft *et al,* 1968). The virus particles were pelleted by centrifugation at 150,000^{X}g for three hours.

Preparation of Viral Proteins

TMV: TMV protein was obtained following dissociation of the virus at pH 9 and removal of the RNA by a passage through a small DEAE ion exchange column equilibrated with 0.12 M Tris hydrochloride,pH 8 (18).

CCMV: CCMV protein was obtained by dissociating the virus at pH 8 and precipitating the RNA with $CaCl_2$. The protein was obtained in a usable form after gradually decreasing the salt concentration to 0.2 M KCl by dialysis (19).

Preparation of Viral RNA

Intact viral RNA was obtained from both viruses by rapid treatment with 50% SDS at 60^{o} C (20). The protein and SDS were removed by the addition of equal volume of 100% (wv) $NaClO_4$. The yield of RNA was usually 30-40% of the RNA content of the viruses.

TMV Assembly in Vitro

TMV particles were assembled in vitro by mixing the nucleic acid and viral protein in a ratio of 3:1 (moles nucleic acid bases:moles protein monomer) in 0.1 M sodium cacodylate buffer, pH 6.9, at a final nucleic acid concentration of 0.5-1 mg/ml (21). Assembly was complete after incubation at 32^{o} for 48 hours, and the assembled nucleoprotein particles were separated by isopycnic centrifugation in a partially preformed CsCl gradient containing 0.1 M ammonium acetate, pH 6.8 (22). The complete particles banded at 1.325-1.330 g/ml. The in vitro assembled particles were concentrated, after removal of the CsCl by dialysis, by centrifugation or dialysis against predialyzed 50% (w/v) polyethylene glycol (MW 20,000, Sigma) in 0.1 M NH_4 acetate, pH 6.8.

CCMV Assemby in Vitro

CCMV particles were assembled in vitro by combining the nucleic acid and protein in a ratio of 1:4-5 (w/w) so that the final concentration of nucleic acid was 0.2 mg/ml. The reaction mixture was adjusted to 1 M NaCl, 0.02 M Tris, 0.001 M EDTA, 0.001 M dithiothreitol, pH 7.4, and then dialyzed for 20 hours at 5^{o} against 500 ml of 0.01 M Tris, 0.01 M KCl, 0.005 M $MgCl_2$, 0.001 M dithiothreitol, pH 7.4 (TKMD buffer) (23). By affixing the dialysis bag to a flared glass tube suspended in the dialysis solution, samples could be withdrawn during the assembly of the particles. To obtain the assembled particles, the mixture was layered on a 5-20% sucrose gradient (RNase free, Grade 1, Sigma) made with TKMD buffer and sedimented five hours at 5^{o} at 100,000 g. The material banding as nucleoprotein particles, when compared to identical gradients containing the virus, was concentrated by dialysis against 50%, (w/v) predialyzed polyethylene glycol in TKMD buffer. The polyethylene glycol was predialyzed against TKMD buffer. The particles were converted to their pH 5 form by dialysis against 0.1 M sodium acetate, pH 5.

Preparation of Spin Labeled Poly A (Sl-Poly A)

Spin labeled poly A was prepared by the method described previously (24), except that 3-(2-bromoacetamido) -2,2,5,5 - tetramethyl -1-pyrrolydinyl oxyl (BrTMPO, Syva Associates) was used instead of 3-(2-iodoacetamido)- 2,2,5,5,-tetramethyl-1-pyrrolydinyl oxlyl. The size of poly rA (Sigma or Miles) used was 5 S_w^{20} or 200 to 300 bases long.

Epr measurments were made on a JEOL MEIX spectrometer interfaced with a Varian C 1024 signal averager and a Data General Nova Computer.

Correlation times for isotropic spectra manifesting motion that is weakly immobilized were calculated from the formalism of Stone and coworkers (25).

$$\tau = C \Delta H_o \left\{ \left[\frac{h_{(o)}}{h_{(+1)}} \right]^{\frac{1}{2}} + \left[\frac{h_{(o)}}{h_{(-1)}} \right]^{\frac{1}{2}} - 2 \right\}$$

where τ = correlation time

ΔH_o = linewidth of central peak in gauss

h = height of peak in arbitrary units

+1,0,-1 = nuclear spin quantum number; +1 is the low field line; -1 is the high field line

C = Constant which is a function of anisotropy of the g and A tensors = 6.4×10^{-10}.

When BrTMPO was reacted with poly rA, the epr line-shape of the spin label changed from that characteristic of highly mobilized to a weakly immobilized situation. The correlation time was calculated (25) to be 2×10^{9} sec.

The reaction of the pyrrolidinyl compound with poly rA is an alkylation reaction in which about one in one hundred adenine bases is modified by the attachment of the spin label group. The extent of labeling (24) was determined from the area of the ESR absorption (spin label concentration) and by the UV spectrum of polynucleotide bases (nucleic acid concentration). BrTMPO would be expected to form covalent linkage with poly rA at the N-1 position of the base (26).

SDS Treatment of Viral Particles

0.3 ml 25% w/v SDS, preheated 65°C, was added to a 1 OD_{260}/ml virus solution (1.2 ml). The mixture was shaken at 65°C for four minutes and the absorption spectra of the dissociated virus compared to the original spectra corrected for dilution by the addition of detergent.

RNase Treatment of Viral Particles

TMV 50 OD_{260}/ml in 0.02 M Tris pH=7.0 and 0.004 m $MgCl_2$ was incubated for one hour at 37°C with 5 units/ml snake venom phosphodiesterase (Worthington) and 500

unit/ml micrococcal nuclease (Worthington). CCMV was exposed to one unit T_2 ribonuclease (Sigma) per 10 OD_{260} /ml virus for one hour at 37°C in 0.1 M sodium acetate, pH 5.

RESULTS

Physical and Chemical Characteristics of Spin-Labeled Nucleic Acids

TMV, CCMV and reconstituted nucleoprotein particles containing sl-poly rA, or viral RNA were characterized by electron microscopy, UV absorption, density in CsCl, and molecular weight. Nucleoprotein particles containing sl-poly rA appear to be morphologically similar to the virus which supplied the capsid protein (Fig. 1 & 2).

Particles assembled using TMV protein with poly rA or sl-poly rA are proportionally shorter (180Ax400 to 1,000Å) than the original virus (180x3,000Å) since the enclosed homopolymer is shorter than the native TMV RNA (Fig. 1b & 1c). Some of these particles appear to be aggregates which form under the conditions used to prepare the grids: high salt due to evaporation at room temperature.

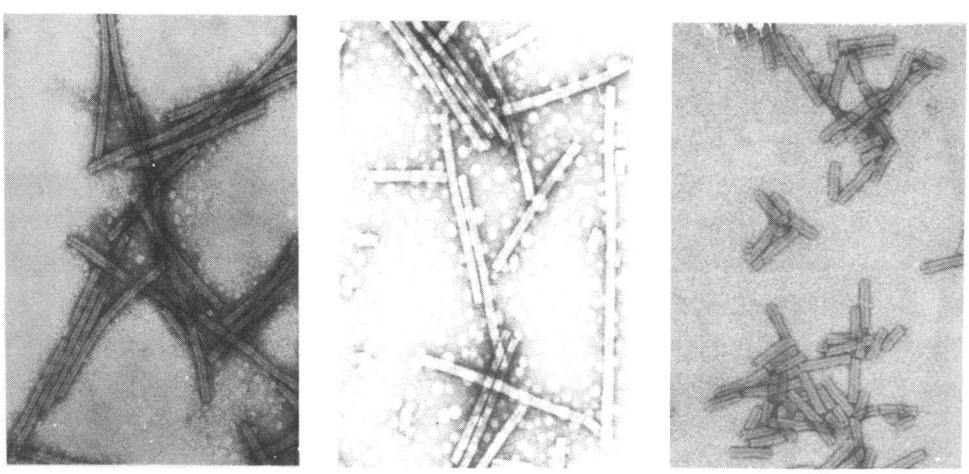

FIGURE 1 a) Electron micrograph of tobacco mosaic virus prepared with 1% uranyl acetate stain on negatively discharged formvar coated copper grid. Approximate magnification 70,000 x. b) Electron micrograph of TMV particle assembled in vitro from TMV protein and TMV RNA. Sample prepared with 1% uranyl acetate stain on negatively discharged carboned formvarcoated copper grid. Approximate magnification 100,000 x. c) Electron micrograph of TMV-like particle assembled in vitro from TMV protein and poly A and purified by ultracentrifugation at 200,000 x g for 12 hours at 5° C. Sample prepared with 1% uranyl acetate stain on negatively discharged carboned formval coated copper grids. Approximate magnification 100,000 x.

Electron micrographs of CCMV show spherical particles which appear to have some surface structure (Fig. 2a). In fresh preparations of the virus, no particles having an electron dense center are seen, indicating an absence of empty shells which can accumulate the stain. The CCMV shown in Figure 2a has a diameter of about 260 Å based on the magnification of photographs. The morphology of CCMV-like particles assembled in vitro from CCMV protein and CCMV RNA ($CCMV\text{-}RNA_{CCMV}$) is identical to the virus. CCMV-like particles containing poly rA are shown in Figure 2b and appear to be identical to CCMV virus.

In order to determine the integrity of the reassembled RNA and poly rA, we determined the sedimentation coefficient both before and after reassembly. TMV-RNA isolated from the virus and from reassembled TMV particles both had identical sedimentation coefficients (S^{20}_w =32). Similarly, both poly rA and sl-poly rA had sedimentation coefficients of 5 S^{20}_w as determined by analytical ultracentrifugation both before and after reassembly.

During the assembly of sl-poly rA into TMV and CCMV-like particles there was no indication of nucleic acid degradation which would be evidenced by a mobile epr spectrum. Therefore, no significant degradation of either viral RNA or the sl-poly rA appeared to occur during the in vitro encapsulation of the polynucleotide by either TMV or CCMV protein.

The TMV virus, and TMV particles reassembled in vitro have densities in CsCl gradients of 1.330 gm/ml which agree with reported values of TMV virus, strain

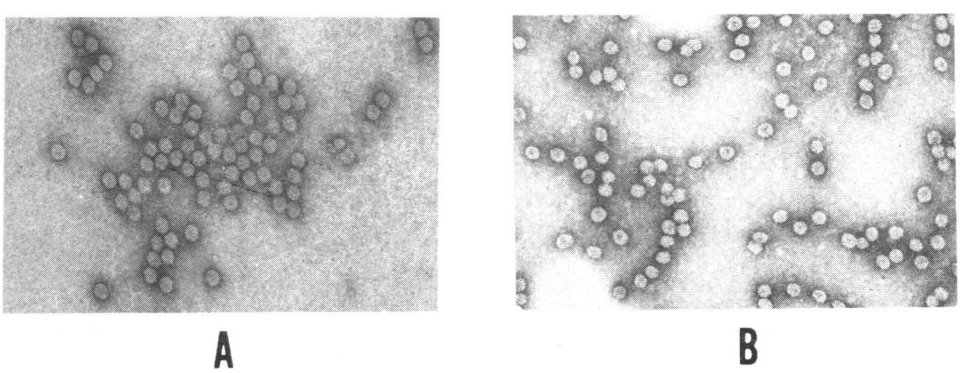

FIGURE 2 a) Electron micrograph of CCMV at pH 5. Sample prepared with 2% uranyl acetate stain on negatively discharged carboned formvar coated copper grid. Approximate magnification 100,000 x. b) Electron micrograph of CCMV-like particles assembled in vitro from CCMV protein and poly A at pH 5. Particles purified on a sucrose gradient at pH 7.4 in the presence of magnesium and then adjusted to pH 5. Sample prepared with 2% uranyl acetate stain on negatively discharged carboned formvar coated copper grid. Approximate magnification 100,000 x.

Ul, of 1.325 gm/ml (2b). TMV-like particles assembled in vitro with poly rA and sl-poly rA band at 1.325 gm/ml. These data suggest that the virus and reassembled particles have similar protein and RNA compositions.

CCMV viral particles have densities in CsCl of 1.356, 1.360 and 1.364 gm/ml (28) while reassembled CCMV particles containing CCMV-RNA have densities of 1.370 gm/ml (23). In our studies reassembled CCMV particles containing CCMV-poly rA form bands with densities of 1.363-1.366 gm/ml in CsCl, again indicating that the composition of these particles are similar.

The TMV preparations used in this study also had a coefficent of 200 S^{20}_w. Viral-like particles containing poly rA appear to have a sedimentation coefficient of about 170 S^{20}_w after the particles were sedimented for twenty hours in a CsCl gradient at room temperature. This value probably reflects the end to end aggregation of the particles.

CCMV has been reported to have a sedimentation coefficient of 80 S^{20}_w (29), or 88 S^{20}_w (12). CCMV-sl-poly rA particles assembled in vitro had a coefficient of 80 S^{20}_w at pH 5 although the preparation did contain some material that had a coefficient of 50 S^{20}_w. The 80 S material probably contains enough nucleic acid to equal that in the intact virus (about 1 x 10^6 daltons). This suggests that the CCMV-poly rA particles contain more than the one molecule of the polynucleotide. The 50 S material is protein aggregated to form empty shells as observed in the electron micrographs of these preparations (Fig. 2b). CCMV protein is known to aggregate to form a 50 S particle which resembles the virus but lacks RNA (30).

The UV spectra of native TMV and TMV-RNA_{TMV} particles assembled in vitro are identical after correction for Raleigh light scattering of the particles in both preparations (Figure 3). TMV-poly rA particles show considerably greater optical absorbance at 260nm. The extinction of poly rA can be calculated to be about 30 OD_{260nm} per mg/ml assuming no hypochromicity in the polymer or 1.2 times greater than RNA at neutral pH and low salt (25 OD_{260} per mg/ml). The maximum absorption of the TMV-poly rA is 1.3 times greater than that of the viral particle so some of the spectral differences between the particles may be a result of the inherent differences in the extinction coefficient between RNA_{TMV} and poly rA.

The spectra of CCMV virus and CCMV viral particles reconstituted in vitro are nearly identical (Figure 4). The CCMV-poly rA particles have slightly greater absorbance at 260 nm. None of the spectra shows significant light scattering. The maximum absorbance of CCMV-poly rA particles is about 1.05 times the absorbance of particles containing viral RNA; the increase may be due to the difference in the extinction coefficient between the RNA and poly rA. The

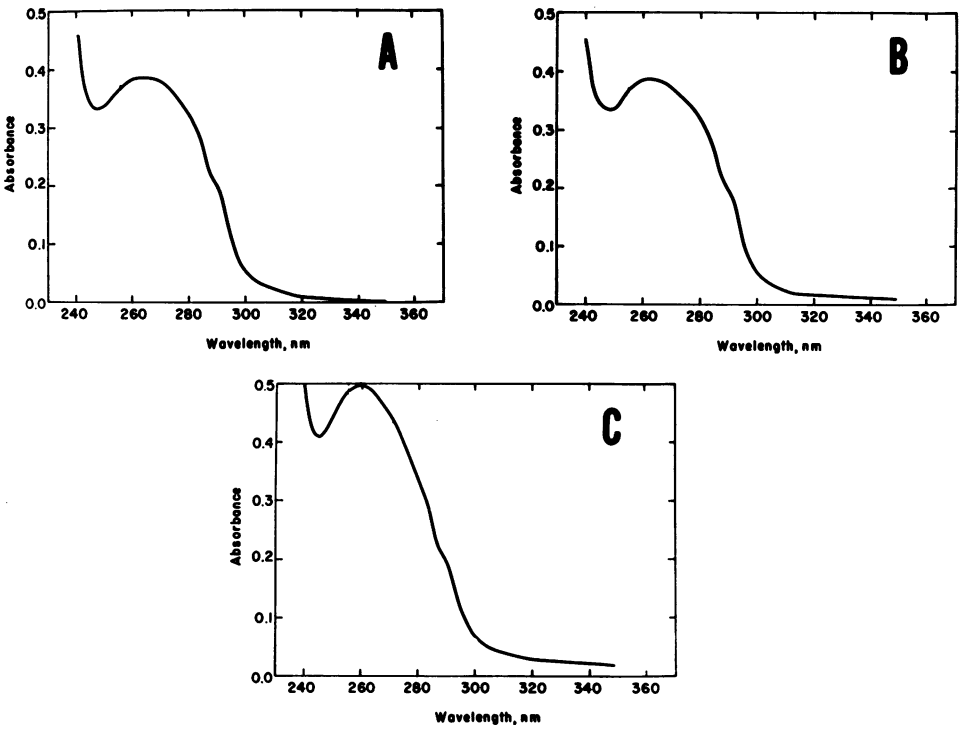

FIGURE 3 UV Spectra of TMV and in vitro Assembled Particles. UV absorption spectra of a) TMV virus, b) TMV particles assembled in vitro using TMV protein and TMV RNA, c) TMV-like particles assembled in vitro using TMV protein and poly A. The spectra are averages of more than size spectra, and are normalized to 0.2 OD_{290}, the data are also corrected for light scattering using the Rayleigh light scattering relationship based on the absorbance of the solutions between 550 nn and 370 nm.

extinction coefficient of poly rA is about 1.2 times greater than that of CCMV RNA. The CCMV-poly rA particle may contain slightly less nucleic acid than the virus so the increase in maximum absorbance would be less than expected. Additionally, some aspects of the structures of the poly rA in the viral particle may differ from the structure of viral RNA in the particle, a result of the shorter length of the poly rA compared to viral RNA or a result of the tendency of poly rA to form double-stranded structures readily at pH 5, the pH at which the viral particle is most stable.

The structure of the nucleic acid inside the original viral particles and the reassembled particles was compared by observing the U.V. spectral changes during assembly as well as disassembly with detergent. The hyperchromic or hypochromic changes of the nucleic acid absorption spectra associated respec-

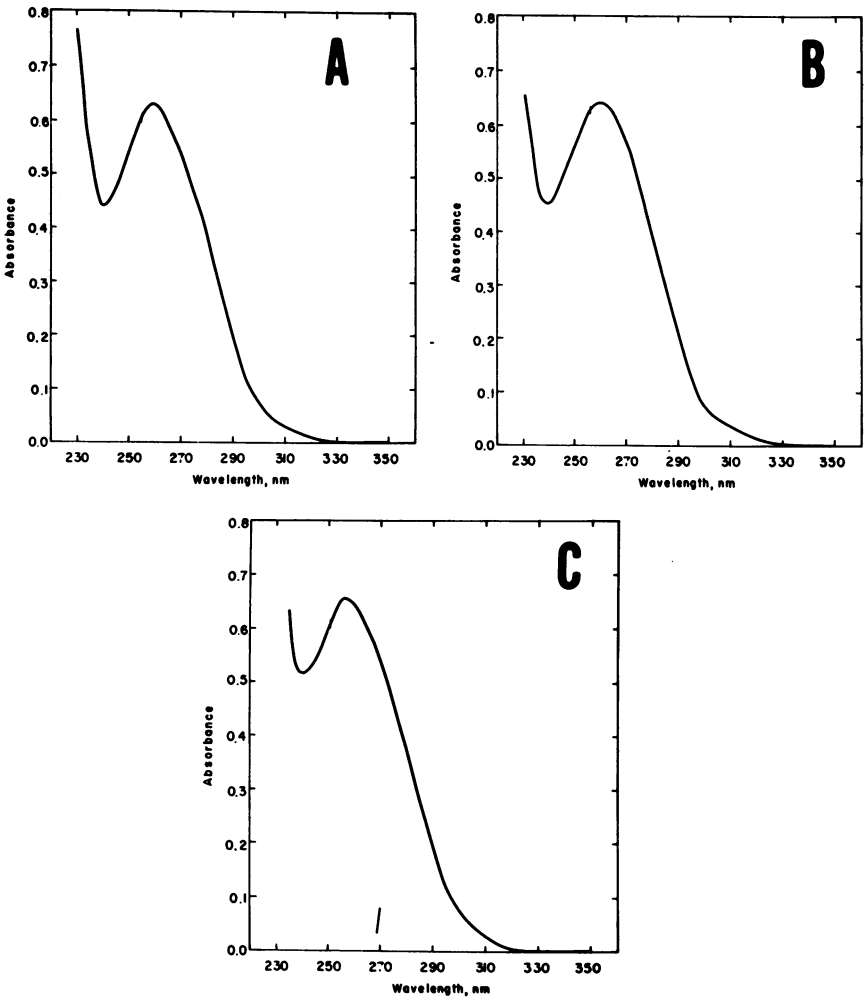

FIGURE 4 UV Spectra of CCMV and in vitro Assembled Particles. UV absorption spectra of a) CCMV virus, b) CCMV particles assembled in vitro using CCMV protein and CCMV RNA, c) CCMV-like particles assembled in vitro using CCMV protein and poly A. All particles are at pH 5 in 0.1 M Na acetate. The spectra are averages of more than size spectra and are normalized to 0.2 OD_{290}.

tively with either the assembly or disassembly of the viral particles. TMV and CCMV particles each appear to have consistent changes in their nucleic acid spectra regardless of the composition of RNA (natural or poly rA) used implying similar arrangement of the nucleic acid in either TMV or CCMV.

Epr Studies

Combining the spin-labeled nucleic acid with purified TMV protein in the

conditions which promote assembly of a virus-like nucleoprotein particle, does not alter the epr signal initially (Fig. 5a). After assembly is allowed to proceed for 48 hours at 32^{o}, the epr spectrum of the mixture is a composite of highly mobile unencapsulated sl-poly rA and highly immobile sl-poly rA (Fig. 5b). The encapsulated sl-poly rA has broader spectral lines than the unencapsulated poly rA. In order to clearly identify its spectral features, the TMV-like particles were purified on an isopycnic CsCl gradient removing all unencapsulated spin labeled nucleic acid. The purified particles have a highly immobilized epr spectrum (Fig. 5c), which represents the immobile component in the composite spectrum before purification. Because of the strongly immobilized nature of the spin label motion (τ = 5 x 10^{-7}sec), the spectrum of TMV sl-poly rA is best characterized by the hyperfine coupling constant. The shape of the spectrum indicated suggests that the motion is slightly anisotopic. The component of the anisotropic hyperfine tensor are $2T_{11}$ = 68.3G and $2T_{\perp}$ = 24G. Since the motion of the spin label attached to the TMV-poly rA nucleoprotein particle was too slow to provide information by conventional epr, we performed preliminary saturation transfer experiments on the system. These experiments indicated that the motion

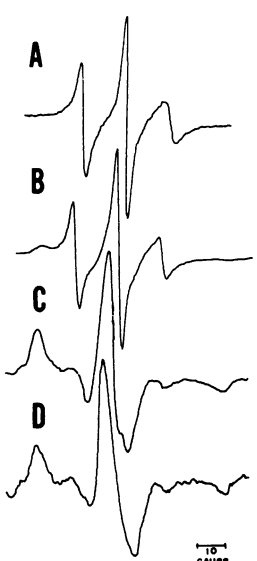

FIGURE 5 ESR Spectra of TMV_P-sl-poly A Particles. a) ESR spectrum of initial mixture of TMV protein and sl-poly A to begin assembly of TMV-like particles. b) ESR spectrum of assembly mixture of TMV protein and sl-poly A after assembly in vitro has proceeded 48 hours at 32^{o} in 0.1 M Na cacodylate, pH 6.9. c) ESR spectrum of TMV_P-sl-poly A after purification on CsCl gradient. d) ESR spectrum of TMV_P-SL-poly A after purification on CsCl gradient and treatment with micrococcal nuclease for one hour at 37^{o}.

of the spin label possessed a correlation time between 10^{-7} and 10^{-6} sec (J. Paxton, W. Caspary, unpublished results).

To establish that the sl-poly rA is enclosed within the viral structure, the particles were tested for sensitivity to RNase. Simultaneous exposure of the particles for one hour at 37° to 5 units/ml (a concentration that causes rapid degradation of sl poly rA) of snake venom phosphoreductase and 500 units/ml micrococcal RNases produced no changes in the epr spectra of the particles (Fig. 5d). Native TMV and TMV reassembled in vitro are resistant to such RNase exposure. In a control experiment, hydrolysis with RNase of the sl-poly rA alone produced the distinctive epr signal of a highly mobile spin label group.

When purified CCMV protein and sl-poly rA are combined using conditions which promote the assembly of virus-like nucleoprotein particles, the initial epr signal of the mixture is indistinguishable from that of the spin labeled nucleic acid alone (Fig. 6a). As assembly of the particles proceeds, the epr signal of the reaction mixture develops a more immobilized component indicating that a

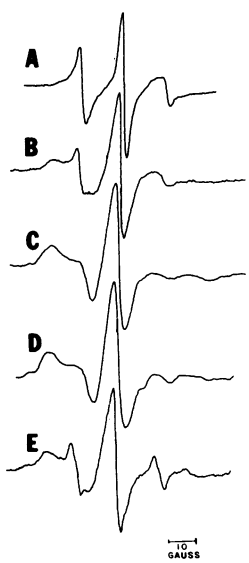

FIGURE 6 ESR Spectra of CCMV-sl-Poly A Particles. a) ESR spectrum of initial mixture of CCMV protein and sl-poly A combined initially in vitro. Sample in NTDE buffer, 1 M NaCl, pH 7.4. b) ESR spectrum of CCMV protein and sl-poly A mixture after dialyzing 20 hours against low salt buffer, pH 7.4, containing 0.005 M Mg^{++} (TKMD). c) ESR spectrum of CCMV-sl-poly A after purification on sucrose gradient in TKMD. d) ESR spectrum of CCMV-SL-POLY A particles after purification and adjustment to pH 5 in 0.1 M Na acetate. e) ESR spectrum of CCMV-sl-poly A in 0.1 M Na acetate pH 5, after exposure to RNase T_2 for one hour at 37°.

fraction of the spin label groups have more restricted motion (Fig. 6b). At the conclusion of assembly, the CCMV nucleoprotein particles are purified on a sucrose gradient. After removal of the sucrose by dialysis, these particles at pH 7.4 in low salt buffer conditions Mg^{++}, produce an epr signal with a correlation time greater than 5×10^{-7} sec. (Fig. 6c). Subsequently, conversion of these particles to their final form at pH 5 does not significantly alter their epr signals (Fig. 6d). The splitting in these spectra is 60.6 G and the line shape indicates isotropic motion.

CCMV-like particles assembled in vitro using homopolynucleotides are not completely resistant to RNase, and particles assembled in vitro with sl-poly rA likewise are sensitive to incubation with 1 unit/ml of RNase T_2 at 37^0 for one hour (Fig. 6e). This concentration of RNase T_2 rapidly degraded sl-poly rA at pH 5. Electron microscopy however, established that these appear to be intact particles.

The sl-poly rA in the initial dialysis buffers used for the assembly of CCMV-like particles in vitro has a correlation time ($\tau = 7 \times 10^{-9}$ sec.) which indicated slight restriction of the motion of these labels. Consequently, the highly immobilized fraction of the spin label population which develops during assembly must be a product of the association between the homopolymer and the CCMV capsid protein. In acetate buffer at pH 5, sl-poly rA alone has a correlation time of 10^{-8} sec, indicating that the group associated with the nucleic acid bases in these conditions is moderately immobilized. Poly rA tends to form base paired helical structures in acid pH conditions (31) and the immobility of the spin label reflects this rigid conformation. The correlation time of the label in the CCMV-like particle containing labeled nucleic acids ($\tau < 5 \times 10^{-7}$ sec.) is somewhat less than the correlation time for the polymer alone in acid conditions ($\tau = 10^{-8}$ sec.), indicating that the additional restrictions of the motion of the group is a product of the association between the nucleic acid and the protein in the virus-like particle.

DISCUSSION:

The purpose of this work is to demonstrate the feasibility of using epr to obtain structural information of nucleic acids in a complex biochemical environment. Our choice of encapsulated viral particles provided us with models for nucleoprotein interactions whose physical properties are understood reasonably well. These models afford a degree of complexity typical of simple biochemical systems.

Our results indicate that sl-poly rA can be encapsulated by TMV and CCMV

protein and that the resultant complexes manifest physical properties similar to TMV or CCMV virions. Our results also show that the epr spectrum of encapsulated and unecapsulated sl-poly rA are distinctly different and that the process of assembly can be monitored readily.

Within the limitations imposed by our ability to interpret highly immobilized spin label spectra by conventional epr it has been possible to describe the motion of the nucleic acid bases attached to spin label groups within two viral particles.

For TMV-poly rA nucleo-protein particles, a preliminary saturation transfer experiment and theoretical calculations indicate that the motion of the spin label and that of the TMV particle appear to have similar correlation times. This indicates that the motion of the spin label is tightly coupled to the motion of the nucleoprotein particle. Based on the dimensions of the virus particles one can calculate the rotational correlation time of the entire virus particle. When observed from a frame of reference outside the virus particle such as the detection apparatus for epr, the spin label group contained in the particle should move at least as fast as the entire particle. Consequently, the correlation time or rotational relaxation time of the virus particle constitutes the lower (slower) limit of the observed correlation time of the encapsulated spin label group. Using the corrected equations that Perrin (32) derived for the rotation relaxation time of prolate ellipsoids, one can calculate a correlation time around each of the axes of a full-length TMV particle. TMV, with dimensions of 180 Å x 3000 Å, has a correlation time around the short axis of 1.1×10^{-3} sec. and around the long axis of 9.6×10^{-5} sec. When one considers the estimated dimensions of a TMV-like particle containing poly rA (180 Å x 300 Å), the correlation time for rotation around the long axis is 4.8×10^{-6} sec. and around the axis perpendicular to the helix axis is 1.2×10^{-5} sec. Since the observed correlation time ($\tau = 5 \times 10^{-6}$sec.) from a preliminary saturation transfer experiment of the spin label in sl-poly rA-TMV approaches the theoretically determined correlation time of the TMV nucleoprotein particle about the long axis ($\tau = 4.8 \times 10^{-6}$sec.) we can conclude that the spin label is rigidly held by the packing forces of the TMV particle.

The positions of the amino acids in the TMV protein monomers (33) and the position of the nucleic acid phosphate group in the TMV viral particle are well defined and observable by X-ray crystallography (2,6). Given the available space, possible locations of the RNA nucleotide bases can be deduced and described. The portion of the protein subunits adjacent to the internal channel of the virion is set in a defined position after interaction with the encapsula-

ted RNA (33), implying that there is little motion of the encapsulated nucleic acid bases as well. The nucleic acid bases are likely to be surrounded by the protein subunits. These data and the fact that RNA in the virion is resistant to RNase attack (34,35) supports a model in which the nucleic acid in the nucleoprotein particle is continuously and completely enclosed.

Saturation transfer experiments were not done on CCMV-poly rA. The diameter of the CCMV-like particles containing poly rA is equal to that of the original virus (260 Å) and the calculated correlation time for rotation of this spherical macromolecule is 6×10^{-6} sec. However, since its epr spectrum was also highly immobilized ($\tau < 5 \times 10^{-7}$ sec), we conclude that the motion of the spin label is controlled by the motion of the whole nucleoprotein particle. Thus the freedom of motion enjoyed by sl-poly rA is lost when bound in this nucleoprotein complex.

If the motion of the spin label is highly coupled to the motion of the nucleoprotein particles, the shape of the particle should affect the anisotropy of the spin label motion. TMV is a rod-like particle and the epr spectrum of this particle is anisotropic. The CCMV particle, which is spherical, manifests isotropic motion when monitored by this epr technique.

The use of exogenous agents such as micrococcal nuclease and RNase T_2 provides an interesting insight into the structure of these nucleo-proteins.

TMV-sl-poly rA is totally resistant to nuclease attack as is the native virion (34,35). This suggests that the internal structure of the TMV-sl-poly rA is not significantly destablized when compared to the native virion; only the lengths of the assembled particles have been changed.

CCMV, on the other hand, appears sensitive to the size of the RNA present. The RNA enclosed is in the intact CCMV nucleoprotein particle is resistant to RNase at pH=5 (36,37). It is thought that the RNA stablizes the viral structure through its reversible association with the capsid protein (38,39,40). In the swollen form (pH=7 without Mg^{++}), CCMV can be cleaved at defined sites by mild treatment with nucleases (28,41). The spin labeled encapsulated poly rA, however, is susceptible to RNase at pH=5. At pH=7 without Mg^{++}, (spectrum shown in Figure 6b contains Mg^{++}) it appears to dissociate completely since the epr spectrum becomes mobile and electron micrographs showed that no CCMV particles were present. This suggests that in contrast to TMV, the internal viral structure of CCMV is critically dependant on the size and perhaps composition of the RNA encapsulated.

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